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6. AUTHOR(S)
Sheryl S. Smith, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Hahnemann University
Broad and Vine
Philadelphia, PA 19102-1192

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13. ABSTRACT (Maximum 200 words)

The olivo-cerebellar circuit plays a role in the coordination of the distal limbs. The present study was proposed to determine the behavior of individual neurons in this circuit, recorded chronically as ensembles of 10-20 during tests of limb coordination across spontaneous changes in limb coordination. Hormone (estrous) and circadian cycles are known to be associated with improvements in the speed and accuracy of limb trajectory, and will be used in this study as a model of changes in performance. Underlying circuit properties which accompany changes in performance will be assessed during performance paradigms. Rats, chronically implanted with microwires in the dorsal accessory olive and arrays of Purkinje cells in the paravermal cerebellum will be monitored during treadmill paradigms employing constant speed, variable acceleration and perturbed gait. Single unit discharge will then be analyzed and correlated with changes in performance associated with hormone state. The following parameters will be assessed: 1. step-cycle correlated discharge, 2. the strength of olivo-cerebellar connections, using cross-correlation techniques, 3. the degree of synchronized olivary oscillatory discharge, a putative timing mechanism for rapid movements and 4. changes in center-surround properties of adjacent arrays of Purkinje cells.

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Technical Report

Sheryl S. Smith, Ph.D.
AFOSR F49620-93-1-0136/DEF

Cerebellar circuit mechanisms which accompany coordinated limb trajectory patterns in the rat: Use of a model of spontaneous changes in limb coordination

Research Objectives

Limb coordination is known to be altered as a function of overall systemic state, such as that associated with endogenous variations in hormones (estrous), circadian state and arousal. In particular, the estrous cycle is associated with significant cyclic improvements in the speed and accuracy of limb movements in terms of hurdle negotiation and in response to variable acceleration of a treadmiull. The goal of this proposal is to quantify electrophysiological activity of the rubro-olivo- cerebellar circuit of the rat associated with these marked alterations in performance state observed across hormone and circadian states.

1. What changes in electrophysiological activity of the olivo- cerebellar circuit are observed during tests of limb coordation?

For this and the following aims, chronically implanted microwires will simultaneously record unit activity from ensembles of individual neurons within the rostral dorsal accessory olivary nucleus (rDAO) and/or Purkinje cells of the intermediate cerebellum. The activity of the circuit will be assessed during challenges to the sensorimotor system using paradigms employing constant speed, variable changes in treadmill acceleration and disrupted locomotion in order to assess motor-correlated activity from the individual components of the circuit. Activity of the Purkinje cell is known to be correlated with the step cycle (stance or flexion phase), while the olive served to signal event changes or "motor error".

2. Can changes in the strength of olivo-cerebellar connections be observed across estrous associated changes in limb coordination, assessed using cross-correlation techniques?

3. Do synchronized olivary oscillations alter across estrous- associated performance state? Rhythmic olivary discharge has been described as a putative internal timing mechanism that facilitates rapid, alternating movements of the limbs, one parameter improved on estrus. Therefore, populations of olivary neurons will be monitored across hormone state to determine the degree of synchronized rhythmic behavior during rapid locomotion paradigms.

4. Do center-surround properties of Purkinje cells change across hormone state? The contrast sharpness of center-surround Purkinje cell activity will also be tested to determine whether resolution of this system improves as limb coordination improves.

94-28335

Research Status

Results

For this protocol Purkinje cells within the paravermal cerebellum were recorded using chronically implanted microwires to allow up to 23 individual single units to be discriminated from one rat. Discharge can then be assessed during treadmill locomotor paradigms across hormone state (estrous cycle). The night of estrus (high circulating levels of estradiol and progesterone) is associated with improved limb coordination.

1. Changes in movement-correlated Purkinje cell discharge across the estrous cycle

Discharge (simple spike) recorded from individual Purkinje cells during treadmill locomotion (tread on) was significantly greater than discharge recorded during the stationary phase (tread off) in 85% of the cells recorded. Both the circadian and estrous cycles were associated with changes in the magnitude of this locomotor-correlated discharge relative to discharge during rest. When the population data is summarized, a number of significant changes can be noted across the estrous and circadian cycles: The maximal locomotor-correlated discharge (trd on) recorded from a Purkinje cell occurred on the night of BE (dark) relative to other days of the estrous cycle ($P<0.05$). In the light, locomotor discharge was greater during proestrus, the day when circulating E_2 levels peak, compared with similar discharge recorded during other phases of the cycle ($P<0.01$). In contrast, the circadian cycle was not associated with alterations in the magnitude of locomotor-correlated discharge recorded on diestrus. Locomotor-correlated discharge was also greater at the faster treadmill speed ($P<0.001$) versus the slower speed, and estrous cycle changes in locomotor correlated discharge were also more apparent when tested in rats run at 11 rather than 4 cm/sec. These results are representative of 43 cells (10 rats, 3-5 cells/rat) for the light phase and 49 cells (10 rats, 2-6 cells/rat) for the dark phase of the light:dark cycle.

Estrous cycle changes in the step-swing cycle

The step cycle can be described as a 5 stage event and for this study is comprised of: footfall, stance, early thrust, late thrust (or flexion) and swing phases. The duration of these step cycle phases was assessed for rats on estrus and diestrus. Results of videotape analysis of behavior indicate that the early and late extensor thrust phases of the step cycle are shortened by approximately 275 msecs when evaluated on the night of BE compared with values obtained on D.

Estrous cycle changes in step cycle-correlated discharge

Purkinje cell discharge recorded during treadmill locomotion in all cases was correlated with either the stance or the late thrust (LT or flexion) phase of the step-swing cycle, as has been described by others (Apps and Lidierth, 1989). The evoked discharge rate of a representative cell averaged around the LT timepoint (± 200 msec) increased markedly on BE (from 70 to 92 Hz) compared with its rate recorded on D, when the rat was run at the faster treadmill speed. In addition, the timing of this evoked discharge was altered, as the cell began to fire 50 msec earlier and decreased discharge 100 msec earlier than when tested on D. In contrast to this finding, a slight decrease in discharge was noted on BE compared to diestrous values when the rat was run at the slower treadmill speed.

When the population data was evaluated, maximal discharge from 90% of the Purkinje cells recorded correlated with the stance phase of the step cycle. When tested at the faster treadmill speed, locomotor-correlated Purkinje cell discharge was seen to increase significantly ($P<0.012$) on behavioral estrus (E), compared to values obtained on D, irrespective of the circadian cycle. In addition, discharge during the swing phase was either lower or not significantly different on E than comparable discharge recorded on D. This differential effect resulted in a greater step cycle modulation associated with an increase in circulating E_2: the step cycle modulation was five- fold greater on E (light) and two-fold greater on E (dark) versus comparable diestrous data. (The step cycle modulation of discharge exhibited significant differences at a confidence level less than 0.05 for all groups except for the diestrous-light data.) In addition, the temporal relationship between increases in Purkinje cell discharge with the onset of the correlated behavior was altered by the estrous cycle: Correlated discharge tended to precede the step cycle correlate (by a mean 130 msec) when recorded on E (dark), compared to D ($P<0.05$).

The slower treadmill speed was associated with significant decreases in both stance-correlated discharge as well as less significant step cycle modulation for estrous rats compared with data obtained at the faster speed ($P<0.0002$). At the slower pace, only estrous rats run in the dark exhibited significant step cycle modulation ($P<0.02$, stance versus swing-correlated discharge). The estrous-dark stance-correlated discharge was signficantly greater than diestrous-dark data by a mean 35% ($P<0.02$); it was also two-fold greater than comparable data recorded in the light ($P<0.02$). However, rats run in the light at the slower treadmill speed did not exhibit significant differences across the estrous cycle. Thus, estrous and circadian cycles are associated with changes in step cycle modulation of Purkinje cell discharge, but this effect is signficantly influenced by the treadmill speed.

Corrective gait response varies across the estrous cycle

The corrective response to gait perturbation (produced by unpredictably varying the treadmill speed) was assessed across the estrous cycle. Both spatial and temporal indices of shoulder displacement were lowest, and thus motor performance was optimal, when assessed during the dark on estrus. At this time, displacement of the shoulder averaged 1.5 (± 0.02) cm. over a 0.5 (± 0.05) sec period during random changes in treadmill acceleration/deceleration. Both parameters were significantly lower during estrus-dark ($P<0.05$) than during the estrus-light (4.3 ± 1.1 cm; 1.85 ± .32 sec) and both diestrus-dark (4.7 ± 1.3 cm; 1.95 ± .42 sec) and diestrus-light (6.6 ± 1.4 cm; 2.4 ± .46 sec) phases. (On diestrus, hormone levels are low during both light and dark.) When assessed during the light, the spatial, but not the temporal displacement parameter, was significantly lower on estrus ($P<0.05$) than on diestrus. In contrast, there were no significant differences between either the spatial or temporal parameters obtained in the light versus the dark on diestrus.

Estrous cycle effects on Purkinje cell discharge recorded during variable acceleration

To assess changes in olivocerebellar activity during gait perturbation the rats were placed on a treadmill whose speed (and acceleration/deceleration) was continuously varied. Most neurons recorded in the cerebellum (39/49) and rDAO (47/59) exhibited increased discharge, relative to both rest and regular locomotion, during this variable treadmill acceleration paradigm. In general, Purkinje cell discharge increased at the time of treadmill onset, and continued to increase to a plateau during the period of variable treadmill locomotion. The termination of treadmill locomotion was accompanied by decreases in Purkinje cell discharge, which declined to a nadir during non- movement (treadmill off). Similarly, olivary discharge was increased during the variable acceleration paradigm.

On the night of estrus, movement-correlated discharge increased to maximal levels of 20 ± 5.0 Hz by the second half of the locomotor period, a 67% higher activity level than the average rate of 12 ± 3.1 Hz, achieved during the locomotor period on the day of diestrus ($P<0.05$). Purkinje cell discharge during non-movement was also greater on estrus ($P<0.05$) than diestrus. In contrast, at treadmill onset, movement- correlated olivary discharge was decreased on estrus versus diestrus (average frequency 3.8 ± 0.7 vs. 7.6 ± 2.0 Hz, respectively, $P<0.05$). Olivary discharge during non-movement was not significantly altered across the estrous cycle.

2. Estrous cycle effects on the strength of olivo-cerebellar cross- correlations

Cross correlation analysis revealed estrous cycle influences on the strength of olivo-cerebellar functional connections. Purkinje cell discharge averaged around the spiking of a rDAO neuron was increased by an average of 30 ± 8.1% on estrus compared with values observed on diestrus ($P<0.05$).

3. Olivary activity across hormone state (light): Variable acceleration paradigm

Use of a variable acceleration treadmill locomotion paradigm (with random alterations in speed and acceleration) produced olivary discharge rates increased by as much as 21% above those obtained during constant speed locomotion or non- locomotion. This increase in discharge during random (and therefore, unpredictable) changes in treadmill speed may reflect the event signal function of the inferior olive, as predictable movement (or lack of movement) increased olivary discharge very little or not at all. Oscillatory activity of this neuronal population is thought to represent an internal timing device which, when synchronized, functions to coordinate activity of a sagittal array of Purkinje cells and can facilitate rapidly alternating movements. Rapidly alternating movements are required by this treadmill paradigm, and performance using this paradigm is facilitated on estrus, following elevations in both E_2 and P. Therefore, this study was designed to investigate influences of these hormones, injected separately or sequentially, on the synchronized oscillatory activity of populations of up to 23 neurons recorded per rat.

Hormone treatment markedly enhanced both the synchronized activity and the oscillatory activity of the population of olivary neurons tested, an effect dependent on the particular steroid(s) injected. On diestrus (low hormone state), 40% of the population of 43 rDAO neurons (assessed in 5 rats) discharged synchronously, with 4 of 43 cells exhibiting a 3 Hz oscillation. These results were obtained during the variable acceleration paradigm, recorded during the light phase of the circadian cycle when performance has been shown to be less than optimal. Progesterone administration (5 µgs, i.p.) increased to 26 the number of synchronized neurons and increased to 18 the number of oscillating neuron. Following treatment with E_2, alone or in combination with P, 90% of the total olivary population was synchronized. However, only combined hormone treatment increased the number of neurons exhibiting a 3 Hz oscillation to 80% (36/43 cells). The results obtained with dual hormone treatment are similar to those observed on estrus (light), following endogenous increases of these hormones in the circulation. Administration of E_2 alone produced effects on oscillatory behavior similar to those observed after administration of P alone. Thus, elevations in circulating estrous hormones are associated with conversion of a non-oscillating, 40% synchronized population of neurons to one in which 80-90% of the population exhibits synchronized oscillations. Synchronized oscillations predominate during hormonal conditions of improved performance which require rapid alternations in limb movements.

Synchronized olivary oscillations across hormone state (dark): Variable acceleration paradigm

This study assessed the number of synchronized, oscillating olivary neurons recorded during the dark phase of the light:dark cycle from rats using the variable acceleration treadmill paradigm. Results were then compared across the estrous cycle. In general, performance on this paradigm is improved when assessed during the dark rather than the light phase of the light:dark cycle. Performance is maximal on the night of behavioral estrus following elevations in both E_2 and P. Thus, this study further investigated the hypothesis that improvements in performance on this task involving rapidly alternating changes in speed are correlated with a higher percentage of synchronized, oscillating neurons within the population tested.

When recorded during the optimal performance state on estrus, 100% of the 43 neurons recorded exhibited synchronized oscillations. On diestrus, 70% of the olivary population recorded during the dark exhibited synchronized oscillations. Thus, this finding supports the hypothesis that there is a correlation between performance and the degree of synchronized oscillatory behavior of olivary neurons.

4. Estrous cycle effects on movement-correlated Purkinje cell discharge: Center-surround properties

Two types of behavior-neuron correlations were observed during the recording of Purkinje cell discharge from the paravermal cerebellum (forepaw receptive field) of a rat during intermittent locomotion on a treadmill. The majority of the cells tested (39/49) exhibited increased discharge (simple spike activity) correlated with the stance phase of the step cycle during treadmill locomotion, as has been described in previous reports from our laboratory (Smith et al., 1989) and others (Orlovsky, 1978; Armstrong and Edgley, 1984; Apps and Lidierth, 1989). The discharge of the remaining sub-population of cells tested was suppressed during treadmill locomotion.

The activity of these two populations of neurons was differentially modified between late proestrus and estrus following elevations in circulating E_2 and P. At this time, the activity of the movement-activated subset of Purkinje cells was increased by an average of 115 ± 10.2%, compared to discharge of the same neurons recorded on diestrus, when hormone levels are comparatively lower. In contrast, activity of the movement-suppressed subset of Purkinje neurons was further suppressed on late proestrus/estrus compared with diestrous values. For the entire sample of 10 neurons (of a total 49) which exhibited movement- suppression during diestrus, a mean 84.6 ± 6.7% increase in suppression was observed on estrus (versus diestrus). Thus, increases in both excitation and suppression of Purkinje cell discharge are observed on estrus, the period of enhanced limb coordination. This may be reflected in an enhancement in the contrast of center-surround properties of arrays of adjacent Purkinje cells. This increase in gain or "contrast" may result in a finer resolution of processing, analogous to the role of contrast enhancemnt in visual perception. A more accurate readjustment of the limb, via gain changes in Purkinje cell discharge may be the physiological outcome.

Publications

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2. Smith, S.S. Activating effects of estradiol on brain activity. In: Berg, G, Hammar, M. The Modern Management of the Menopause, New York: The Parthenon Publishing Group; pp. 279-294, 1993.

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4. Smith, S.S. and Chapin, J.K. Dynamic changes in the olivo- cerebellar circuitry are associated with improvements in performance observed across the estrous cycle of the rat. J. Neurosci. (submitted).

5. Smith, S.S. Female sex steroids: From receptors to networks to performance - Actions on the sensorimotor system. Progress in Neurobiology (in press).

6. Lee, R.-S., Smith, S.S., Chapin, J.K., Shimizu, N., Waterhouse, B.D., Maddux, B.N., Woodward, D.J. Effects of systemic and local ethanol on responses of rat cerebellar Purkinje neurons to iontophoretically applied norepinephrine and GABA. Brain Research (in press).

7. Lee, R.-S., Smith, S.S., Chapin, J.K., Waterhouse, B.D., Shimizu, N., Maddux, B.N., Woodward, D.J. Effects of systemic and local ethanol on responses of rat cerebellar Purkinje neurons to iontophoretically applied GABA. Brain Research (in press).

Participating Professionals

1. John K. Chapin, Ph.D. (collaborator) Professor, Dept. of Anatomy, Hahnemann Univ., Philadelphia, PA 19102-1192.

2. Jun Li (student) Ph.D. program, Inst. for Neuroscience

3. Miguel Nicolelis, Ph.D. (collaborator) Assistant Professor, Dept. of Physiology, Hahnemann Univ.

4. Valerie Haftel (student) Masters program in Physiology, received M.S. in July 1994.

5. Ronald Markowitz, M.A., Research assistant.

Presentations

1. Smith, S.S. Synchronized olivary oscillations are triggered by the neurosteroid pregnenolone. Soc. for Neuroscience 1993, Washington, D.C.

2. Smith S.S. Activating effect of estradiol on brain activity. Chair, symposium: Hormones, mood and behavior: The VII International Congress on the Menopause, Stockholm, Sweden, 1993.

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5. Seminar presentation: Caltech, lab of James Bower, Ph.D., March 1994.

6. Smith, S.S. Steroid effects on olivo-cerebellar circuits, Symposium: Hormonal and non-hormonal effects of steroids in the CNS (R. Olsen, chair), Albuquerque, New Mexico, March, 1994.

7. Seminar presentation: NYU Medical School, Dept. of Physiology, April 1994.